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Myotonic Dystrophy Type 1 Diagnostics: A Changing Trend

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***Abstract**: Myotonic dystrophy type 1 or Steinert's disease is the most common form of adult muscular dystrophy affecting multiple systems with a wide spectrum of phenotypes. This paper focuses on the different methods to diagnose myotonic dystrophy type 1 that have been used over the years and the various modifications that have been made to the previous diagnostics. The different diagnostic assays and their different advantages and disadvantages are discussed.*
***Keywords:** Myotonic Dystrophy Type 1, Diagnostics, Method*

I. Introduction

1.1. **Prevalence:**Myotonic dystrophy or dystrophia myotonica (DM) is a group of disorders affecting multiple systems with a wide spectrum of phenotypes. It is an autosomal dominant condition which may be congenital or adult in onset. Myotonic dystrophy type 1 (DM1) or Steinert's disease is the most common form of muscular dystrophy in adults, with a prevalence of 1 in every 8,000 individual worldwide[1].However, DM1 is less prevalent in certain areas of Japan (1:100,000), Asia (1:18,000), and Iceland (1:10,000), and even rarer among African blacks [2].

1.2.Gene Location: In case of DM1, the defect in the gene is localized to the long arm of chromosome 19 from 19q13.2-13.3 encoding a protein kinase known as myotonin protein kinase coded by the myotonic dystrophy protein kinase (*DMPK*) gene. *DMPK* is the only gene found to be associated with DM1[3][4]. The trinucleotide repeat is located in the 3` untranslated region of *DMPK* gene and has an altered number of CTG trinucleotide repeat. The severity of the disorder being directly correlated to the number of repeats [2][5].

1.3.Mechanism: The number of CTG repeat is highly polymorphic in both normal and affected individuals. A normal individual possesses alleles with the number of CTG repeats ranging between 5 and 35, whereas an affected individual showing clinical signs of myotonic dystrophy has CTG repeats varying from 50 to several thousand in number. The alleles which fall in the range of 36 to 49 CTG repeats are without any known clinical symptoms and are hence considered as 'Premutations' having the potential to expand during gametogenesis. As can be seen in Table 1, more the number of CTG repeats in affected individuals more severe is the disorder. The stability of transfer of the repeat is also affected causing a larger number of repeats being transferred to the offsprings[5][6][7][8].Thus as the number of CTG repeat units increases in the *DMPK* gene, it also causes an earlier onset of the disorder in the later generations within a family. This is known as 'Anticipation' thus linking the phenotypic severity of the disease with the age at which it occurs in a particular individual[9].

1.4. Classification

Table1: Classification based on number of repeats

CTG repeats	Phenotypic severity	Stability
5-35	Normal individuals with no phenotypic features	The repeats are Stable
36-49	Permutations showing no phenotypic features	They may or may not be stable
50-150	In lower number may not show any phenotypic features or may show features of mild or classic DM1	These are unstable
Over 150	Shows high phenotypic features of classical, congenital or juvenile DM1	These are unstable

The classification of DM1 can be done clinically into four different types:

1.4.1. Mild DM1: This type of myotonic dystrophy is composed of mildly affected individuals who and may present with premature cataracts as the sole clinical features. There is a chance of myopathy and abnormalities in cardiac conduction which may have a late onset. The myotonia in this case can be detected by electromyography.

1.4.2. Classic DM1: It is also known as 'Adult onset' DM1 that may occur in the twenty's or thirty's of an individual's life with weakening of distal bones being the most common symptom involving the flexors of the arms and legs (fingers) affecting the grasping capability of the hands and balance of the body. In addition, a patient may also present with the conditions which occur in the case of Mild DM1. They may also suffer from gastrointestinal symptoms like abdominal pain, diarrhea, constipation etc and fatigue affecting the quality of life of an individual.

1.4.3. Juvenile DM1: It is similar to the mild and classic forms of DM1 mentioned above in the phenotypic features, but in this case which is a more severe form as compared to the other two an affected individual may also suffer from behavioural and cognitive abnormalities and may have difficulty when it comes to either learning in school or when it comes to socializing.

1.4.4. Congenital DM1: One of the symptoms in this case may be seen as early as when fetal development is monitored in the form of an excess amniotic fluid being present in the developing fetus and poor fetal movement which can be seen before the birth of an infant with congenital DM1 of an affected mother. The infant presents with severe respiratory disorder, a weak facial and jaw muscle and hence having feeding and suckling difficulty. The infants may also present with clubbed feat, muscle contractions and a delay in attaining the developmental milestones with mental retardation being very common. There is a very high infant mortality rate. The absence of myotonia makes it difficult to detect even via electromyography. It does not present any similarities with the muscle biopsies found in classical DM1 thus a confirmation can only be made by DNA analysis[1][6].

II. Molecular Diagnosis of Myotonic Dystrophy type 1

The molecular diagnosis of DM1 is a definitive way to confirm the number of CTG repeats, it is a more potent way to confirm the clinical diagnosis. The method of molecular diagnosis is very diverse and attempts have been made over the years to make the method of molecular diagnosis more efficient and effective hence a lot of modification to the original method exists. A brief overview of some of the methods has been discussed below

2.1. Southern Blot Analysis The method of southern blotting has been a very important diagnostic tool for the DM1 cases. It has undergone various modifications over the years in order to increase the detection efficiency. It is broadly divided into two types:

2.1.1. Southern Blotting of Genomic DNA: In this case, the genomic DNA is digested with appropriate restriction enzymes (*BamH*I, *EcoR*I, *Nco*I or *Bgl*I). This technique has proved to be of great importance for the detection of *DMPK* alleles of size of 100 CTG repeats and above [10] . Although the conventional method is useful in the detection of the large CTG repeats in the case of DM1 individuals but it becomes ineffective for small size CTG repeats. Moreover it is a very time consuming and a laborious process which requires a large amount of high molecular weight genomic DNA rendering it unsuitable for automation [11][12].
As shown in appendix, modifications have been done to increase the resolution and quality of the results with the use of field inversion gel electrophoresis (FIGE)[13] or pulse field gel electrophoresis (PFGE)[14]. Breaking the high molecular weight DNA into smaller pieces can be achieved by incubating the gel in HCl before transferring to the nylon hence increasing the efficiency of transfer [12].

2.1.2. Southern Blotting of long range PCR products: This technique requires specific conditions to amplify large fragments with the appropriate normal and affected samples followed by the addition of an end labelled probe[15]. This process is similar to Triplet Primed-PCR in the case that duplicated or deleted repeats in a sequence may remain undetectable. In this case the expanded repeats will appear as smears or multiple fragments [16].

2.2. PCR based Diagnostics: Since its advent as a tool for molecular diagnosis a constant effort has been made to improve the efficacy of this method and hence several modifications of it exist. An attempt has been made to summarize the various PCR methods that have been used over the years with the various concentrations and the altered conditions used while doing the modifications and compiled as shown in appendix.

2.2.1. Conventional PCR: This is a very simple and fast process that can be used to detect of smaller expansions in milder cases of DM1 individuals with CTG repeats below 100 which is very difficult to detect via Southern Blotting however it fails to detect larger repeats and is not preferred for direct diagnosis. Thus it is used generally in the detection of repeats pertaining to normal individuals and small expansions. But a very distinct advantage of this process is that the detection range can be extended to cover a slightly larger number of

CTG repeats by using special additives which can be seen in appendix [2][17][18]. In another way the CTG repeats can also be detected and characterized rapidly by the use of primers which are fluorescently labelled and made to flank the CTG-repeat region and the analysis of the PCR product can be done by capillary electrophoresis [16] [19].

2.2.2. Small Pool (SP) PCR: This PCR technique is used less frequently and is used with Southern Blot Analysis to give smeared or diffused bands by the amplification of restriction digested and diluted genomic product. With the aid of auto-radiography and oligo specific hybridization techniques it can detect a CTG repeat size of up to 1300. This makes it a very lengthy process which being time consuming when it comes to analysis [20][21]..

2.2.3. Long range PCR: Long range PCR assays have been found to improve the amplification of the PCR products. They give a higher resolution in sizing of CTG repeats as compared to the conventional PCR being in the range of 800-1700 CTG repeats after direct visualization on gels, this can be further increased to 2700-3700 CTG after transferring to nylon membrane or using oligo specific probe. Thus Long range PCR is coupled with Southern Blot Analysis to give better resolution. This is a simple and rapid process with an effective range that can be increased just like in the case of conventional PCR by the use of special additives. To add to the various sets of primer combinations which flank the repeat regions they also make use of various modifications of the reagents which include special DNA polymerases, glycerol, PCR buffers, Taq stabilizers, GC-enhancers, dimethyl sulfoxide (DMSO) and 7-deaza GTP in case of dGTP are some of the modifications which are listed out in the appendix[22][23][24][25].

Different names have been given to PCR on the basis of their functional modification of the conventional types like Hot Start PCR, which follows a modified procedure from a conventional PCR of manually heating the reaction mixture before adding polymerase. This is done to prevent the nonspecific amplification that takes place at lower temperature[24], Heat Pulse Extension (HPE) PCR is another PCR which is done to increase the amplification efficiency over repetitive and GC rich sequences [26] or Nested PCR which is another modified form of PCR which is done to reduce non-specific binding and uses two sets of primers and PCR product of the first reaction being the template for the second reaction [27].

2.2.4. Triplet-repeat Primed (TP) PCR: As the name suggests, it is a PCR which makes use of three primers in which one lies outside the CTG repeat and one within the repeat known as the 'triplet primed primer' which is added in limiting amount in comparison to the other primer and a primer known as the 'tail primer' which has a sequence complementary to the 'triplet primed primer' and is labelled with a fluorescent dye [28]. The result that is generated by a TP-PCR is a mixture of PCR fragments of different sizes and hence gives a smear on the gel and capillary electrophoresis has been found to be a useful tool for analysis. This is a very useful tool as a wide range of expansions can be detected but due to the signal going off in higher sizes hence it is not a reliable tool for knowing the length information. TP-PCR also fails to detect expansions in the presence of rare interruptions thus in order to exclude a false negative result a bidirectional TP-PCR Southern Blotting is used [29][30][31].

The method of analysis in case of TP-PCR has been modified over the years to make it simpler, reproducible with the reliability of the results. The coupling of it with capillary electrophoresis has made it easier to attain the exact number of CTG repeats by counting the number of peaks. In addition to the methodological advantages of this making fast generation of the results capillary electrophoresis makes the process almost completely automatic and reduces the time of requirement from a week in case of Southern Blot Analysis to as less as three hours in case of TP-PCR which can be a crucial time when it comes to prenatal diagnosis[11].

2.2.5. Quantitative fluorescent (QF) PCR: Quantitative fluorescent (QF) PCR is one of the most recent recruits in the diagnosis of DM1. Having a close association with multiplex PCR it uses three sets of primer each flanking to the either side of the region of interest of *DMPK* gene and to the gene of interest and the reaction is performed together for the three sets in one multiplex reaction with one primer from each of the three sets of primers being fluorescently labelled with the same fluorescent tag for detection. The method though very useful is generally used in the detection of the CTG repeats below 100 and in order to increase the effective range another step needs to be added. This is a useful technique to know the zygosity of the allele to know if the allele is homozygous or heterozygous in nature hence better diagnosis. The selected markers used for reference should not contain deletions or duplications [25][32]. This is a particularly useful technique in case of preimplantation genetic diagnosis (PGD) for DM as it applies the use of polymorphic markers like D19S112 and APOC2 [24] or

APOC2 and D21S1414 which are made to amplify with the *DMPK* gene thus providing in diagnosis and hence allowing contaminants to be detected which are a serious problem [33].

2.3. In situ Hybridization Technique: This is a type of hybridization technique which makes use of labelled probes in order to detect a particular target sequence. It is particularly useful technique for the detection of CTG repeat size of over 100 but is a time consuming process that requires highly skilled labour. Long PCR is a very useful technique but has limitations in interpreting results in the case of somatic mosaicism and in cases where the expansion size exceeds the PCR amplification range. To overcome these problems RNA fluorescence *in situ* hybridization (RNA-FISH) has been found to be very useful. It is used to detect the presence or absence of nuclear foci of the expanded trinucleotide repeat in the interphase nuclei of the trophoblastic cells in suspected DM1 fetuses [34][35].

Table 2: Summarization of the advantages and disadvantages of various techniques

S.No.	Technique	Modification	Advantages	Disadvantages	References
1.	Polymerase Chain Reaction (PCR)	Coventional	-Simple & fast -Detects small CTG repeats which are not detectable by SBA -Effective range can be increased by the addition of special additives	-Can only detect small sized repeats. -Effective for CTG below 100 repeats.	Brunner *et al*., 1992; Kim *et al*., 2008; Prior, 2009; Bachinski *et al*., 2009; Kamsteeg et al., 2012
		Small Pool	-Resolves discrete bands when used with SBA -Effective range can be extended to 1300 CTG repeats	-Laborious & time consuming -Visualization of larger repeats requires an association with SBA and hybrisation techniques	Monckton *et al*.,1995; Wong *et al*., 1995
		Long range	-Simple & fast -Effective range between 800-1700 CTG repeats - Effective range can be increased by the addition of special additives and to around 3700 CTG repeats using hybridization technique	Attaining higher resolution by hybridization techniques makes the process lengthy and time consuming	Cheng *et al*., 1996; Brugnoni *et al*., 1997; Kakorou *et al*., 2007; Skrzypczak-Zeilinska *et al*., 2009; Orpana *et al*., 2012
		Triplet repeat primed	-New modifications have made it reliable -Robust -Fast & Simple -Sizing for the presence or absence of alleles	-Sensitive to sequence deletion or duplication -Confirmation is needed hence followed by SBA or done as a bidirectional assay	Falk *et al*., 2006; Musova *et al*., 2009; Braida *et al*., 2010; Radvansky *et al*., 2010
		Quantitative fluorescent	- Very useful method in PGD for DM1 -Detects the zygosity of the allele -Use polymorphic markers to identify contaminats	-Cannot be used to detect extensions over 100 repeats. -Multiplex PCR for better diagnostics	Skrzypczak-Zeilinska *et al*., 2009; Piyamogkol *et al*., 2001; Kakourou *et al*., 2007; Radvansky *et al*., 2010
2.	Southern Blot Analysis (SBA)	SBA of Genomic DNA	-Detects expansion size of over 100 CTG repeats	-It is a laborious and a time consuming process -Not suitable for the detection of small size repeats -Requires large amount of high molecular weight genomic DNA	Falk *et al.,* 2006; Carson *et al*., 2009
		FIGE, PFGE and gel incubation in HCl prior to transferring	Increases quality and resolution and increases the efficiency of transfer of genomic DNA	-It is a time consuming and laborious process	Bachinski *et al*., 2003; Jakubiczka *et al*., 2004; Carson *et al*., 2009
		SBA of Long range PCR product	It increases the sensitivity for the detection of large CTG repeat	- It is a time consuming and laborious process -It is very sensitive to sequence deletion or duplication.	Gennarelli *et al*., 1998; Kamsteeg *et al*., 2012

3.	In situ hybridization		-Identifies expansions at the level of RNA. -It detects sizes above 100 CTG repeats	- It is a laborious and a time consuming process -It gives no information about the size of the expansion	Liquori *et al*., 2001; Bonifazi *et al*., 2006

III. Conclusion

The aim of this paper is to present a wide range of diagnostics for myotonic dystrophy Type 1 diagnostics that has been used over the years. Thus various modifications exist to the conventional methods that were started initially. The improved techniques offer better and faster diagnostics covering a wider range of CTG repeats but also have their own drawbacks hence it is best that a particular assay should be selected as per the experiment requirements like knowing the affected allele, zygosity of the alleles, the size of the expanded repeats that needs to be detected or if the information about the number of repeats is required. As listed in Table 2 some of the conventional methods are particularly useful in case of detecting the lower CTG repeats that occur in case of normal patients or patients with mild DM1.

On the other hand some of the techniques are useful only in the detection of larger CTG repeats like Southern Blot Analysis, which is ineffective for shorter repeat lengths. The other point that needs to be taken into consideration is the time taken to generate the result after successful analysis which can prove to be very important in case of prenatal diagnosis in which time can be a crucial factor. The time taken and the effort have been successfully reduced by the introduction of PCR based diagnostics. In order to make PCR based diagnostics more robust, reliable and accurate it is generally suggested to couple this diagnosis with another test to check for the accuracy. Thus in case of TP-PCR the diagnostic is generally done bidirectionally. The use of QF-PCR and Multiplex PCR has although made the PCR based diagnostics a little tedious but this has increased the accuracy of PCR based diagnosis as it identifies the presence or absence of expanded alleles apart from the sizing aspect and with the use of polymorphic markers it has made this method more reliable as it can identify any contaminants. The use of in situ hybridization technique is more or less secondary when the prior methods are not conclusive or if further validation is needed.

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Appendix: Summary of the various PCR based techniques with conditions

References	PCR Conditions			
	Primers	**Master Mix**	**Cycling temperature**	**Comments**
Brook *et al.* (1992)	96forward:(GGTGCGTGGAGGATGGAACACGGAC) 103reverse:(CCAGTTCACAACCGCT CCGAGCGTG) 98reverse:(GCGTGCGAGTGGACTAACAACAGCTG) 100forward:(CACGCTCGGAGCGGTTGTGAACTGG) 101forward:(CTTCCCAGGCCTGCAGTTTGCCCATC) 102reverse:(GAACGGGGCTCGAAGGGTCCTTGTAGC)	Total-10µl 50mM/l KCl,1.5mM/l $MgCl_2$,10mM/l Tris HCl [pH8.3], 200µM/l dNTP,1µM each Primer,20ng DNA. In case of Radiolabelled experiments oligo101 was incubated (30mins,37°C) with T4 polynucleotide kinase (3µ) in 20µl mix with 50mM/ l Tris HCl, 10 mM/l $MgCl_2$, 5mM dithiothreitol, 0.1mM spermidine and 1µl of (γ^{-32}P) ATP(3000ci/mM)	94°C×3min 35 cycles: [94°C×10sec, 62°C×30sec, 72°C×30sec] 72°C×5min	Flanking PCR was performed on patient samples using three sets of primers with primer combination 101 and 102 giving the best results
Goldman *et al.* (1994)[36]	Used Primer101 and 102 (Brook *et al.* ,1992)	Total-25µl 10mM/l Tris HCl, [pH8.8],50mM/l KCl, 1.5mM/lMgCl 0.1% Triton X,200µM dATP, 200µM dTTP,200µM dGTP,2.5 µM dCTP,50ng of Primer,20-30nCi (α-^{32}P) dCTP,2U Taq polymerase,100ng DNA	35 cycles: [94°C×1min, 66°C×1min, 72°C×1min]	A flanking PCR was performed on 210 unrelated patients to determine the size and distribution of CTG repeats
Hojo *et al.* (1995)[37]	Used Primer101 and 102 (Brook *et al.* ,1992)	Total-25µl 200µM/l dATP,200µM/l dCTP,200µM/l dGTP, 200µM/l dTTP,50pM primers,1U Taq polymerase, 20ng DNA	94°C×3min 35 cycles:[94°C×1.5min, 62°C×1min, 72°C×2min] 72°C×5min 4°C forever	Flanking PCR was performed coupled with Southern Blot to show that DNA analysis is a useful tool in prenatal diagnosis
Guida *et al.* (1995)[38]	Forward Primer : (5`-GCTCG AAGGGTCCTTGTAGCC-3`) Reverse Primer: (5`-GGGGG TGCGTGGAGGATGGAA-3`)	Total-50µl 3mM/l $MgCl_2$, 67mM/l Tris HCl [pH8.3],16.6mM/l ammonium sulphate,6.7µM EDTA,10mM/l 2-mercaptoethanol, 50mg/l BSA,100g/l DMSO, 300nM/l dNTP, 3U Taq polymerase,150ng Primers, ~1µg DNA	95°C×5min 10 cycles: [95°C×1min, 68°C×1min, 20 cycles: 62°C×2min, 72°C×5min] 72°C×10min	The reverse primer used is as per Mahadevan *et al*(1992) and the forward is primer is designed A molecular protocol was developed using flanking PCR for the analysis of normal patients followed by the Southern Blot for larger repeat size.
Meiner *et al.* (1995)[39]	Used Primer101 and 102 (Brook *et al.* ,1992)	Total-50µl 2mM/l $MgCl_2$,67mM/l Tris HCl [pH8.8],16.6mM/l ammonium sulphate,6.7µM EDTA,10mM/l beta-mercaptoethanol,200µM/l dNTP,1U Taq polymerase, 50pM Primers,200ng DNA	94°C×5min, 30 cycles: [95°C×90sec, 60°C×90sec, 72°C×3min] 72°C×7min	Direct method of analysis is used to analyse patients of German population using flanking PCR.
Cheng *et al.* (1996)	DMK9003forward:(5`-CACAGGCTGAAGTGG CAGTTCCA -3`) DMK11111reverse: (5`-TGTCGGGGTCTCAGT GCATCCA-3`)	Total-50µl 1XPCR buffer,1.5mM/lMg(OAc)$_2$,10mM/l Tris HCl, 50mM/l EDTA,0.2mM dATP, 0.2mM dCTP, 0.2mM dGTP,0.2mM dTTP,7-deaza dGTP 1U Taq polymerase,15-50ng DNA	95°C×15sec Mg(OAc)$_2$ or 94°C×45sec, 24 or 33 cycles: [95°C×(10-12)sec, 66°C×6min, 67°C×6min] 68°C×(5-10)min	In case of hot start PCR with "bulk" DNA reactions Mg(OAc)$_2$, was held till the temperature reaches 78°C and for low copy reactions Ampli WAX™ was used. This method made use of flanking PCR to analyze large CTG repeats in the case of Myotonic Dystrophy hence making the diagnosis simpler.
Nanba *et al.* (1996)[40]	Used Primer101 and 102 (Brook *et al.* ,1992)	Total-20µl 1.5mM $MgCl_2$,50mM KCl,10mM Tris HCl, 10% DMSO,200mM dNTP,1U Taq polymerase, 20pM Primers,250ng DNA	- 94°C×3min, 30 cycles: [94°C×1min, 65°C×1min, 72°C×1min]	The prenatal diagnosis was performed using Non-radioisotopic PCR method of analysis.

			72°C×5min	
Warner *et al.* (1996)	For 3`end: P1R:(5`-AGAAAGAAATGGTTCTGTGATCCC -3`) P3R:(5`-TACGCATCCCAGTTTGAGACG-3`) P4CTGF:(5`-TACGCATCCCAGTTTGAGACG T GCTGCTGCTGCTGCT-3`) For 5`end: P2F:(5`-GAACGGGGCTCGAAGGGTCCTTG TAGCCG -3`) P4CAGR:(5`-TACGCATCCCAGTTTGAGACG CAGCAGCAGCAGCA-3`) P3R: (5`-TACGCATCCCAGTTTGAGACG-3`)	Total-25µl 1.5mM/l $MgCl_2$,10mM/l Tris HCl,50mM/l KCl, 10% DMSO,200µM dATP,200µM dCTP, 200µM dGTP,200µM dTTP,2U Taq polymerase, 200ng-1µg DNA For 3`end: P1R(5`fluoresceinated 1µM/l),0.1µM/l P4CTGF, 1µM/l P3R, 1µM/l P2F, 0.1µM/l P4CAGR and 1µM/l P3R	94°C×4min 30 cycles: [94°C×1min, 60°C×1min, 72°C×2min] 72°C×10min	Used Triplet primed-PCR method by using fluorescently labelled primers flanking the repeat region enabling rapid identification of repeats.
Brugnoni *et al.* (1997)	PCR with glycerol: 409-F:(5`-GAAGGGTCCTTGTAGCCGGGAA-3`) 406-R:(5`-CCGTCCGTGTTCCATCCTCC-3`)	Total-50µl 1.5mM $MgCl_2$,50mM KCl,10mM Tris HCl, 0.1% Triton X-100,200µM dNTP,2U DynaZyme DNA polymerase,1 mM Primers,500ng DNA	30 cycles: [94°C×1min, 58°C×1min, 72°C×3min]	In order to improve the analysis of large CTG repeats otherwise not polymerized by PCR for the amplification of CG-rich regions rTth DNA polymerase XL or 10% glycerol was added hence making analysis of large repeat size possible.
	PCR with rTth DNA Polymerase, XL (extra long): 409-F & 406-R primers were used.	Total-100 µl 20 µl XL Buffer II (containing Tricine, potassium acetate, glycerol &DMSO),200µM dNTP, 3.2 µM Primers,1.25 µM $Mg(OAc)_2$,Water-40 µl,4U rTth DNA pol,40 µl (1µg/40µl) DNA	28 cycles: [94°C×20sec, 62°C×7min, 62°C×7min] 72°C×10min	
Sermon *et al.* (1998)[41]	Used Primer101 and 102 (Brook *et al.* ,1992)	Total-50µl 2mM $MgCl_2$,50mM KCl,100mM Tris HCl (pH8.3), 10% DMSO,0.1mg/ml gelatine,0.2mM dNTP, 1.25U Taq pol,1 µM Primers	96°C×5min, 25 cycles: [96°C×30sec, 65°C×30sec, 72°C×30sec] 72°C×6min	The PGD for myotonic dystrophy is done here. The fluorescent PCR was compared with convetional PCR and was found to be more efficient and accurate.
	For Fluorescent PCR Primer 101 was fluorescently labelled.	Total-25µl 3 µl of the PCR product was used as the template and the concentration was kept same except: 1.5mM $MgCl_2$,No DMSO,0.2 µM Primers,	96°C×5min, 10 cycles: [96°C×30sec, 65°C×30sec, 72°C×30sec] 72°C×5min	
Piyamongkol *et al.* (2001)	Used Primer101 and 102 (Brook *et al.* ,1992) APOC2 (Weber & May *et al.*, 1989) or D21S1414 (Sherlock *et al.*, 1998)	Total-25µl 1×Ampli Taq Buffer(10× contains 100mM Tris HCl pH8.3, 500mM KCl, 15mM $MgCl_2$),200µM primers 0.2mM dNTP,1.5 U Ampli Taq Gold™ (PE Applied Biosystems)	94°C×12min, 40 cycles: [94°C×45 sec(96°C for 10 cycles), 60°C×45sec, 72°C×1min,]	The protocol used multiplex PCR for the PGD diagnosis and used APOC2 and D21S1414 as markers.
Falk *et al.* (2006)	Warner *et al.,* 1996	Total-25µl 1.5mM/l $MgCl_2$,1% DMSO,1U Taq polymerase, 100nM/l P1R/P2F 20nM/l P4CTGF/ P4CAGR 100nM/l P3R,500ng-1µg DNA	94°C×12min 30 cycles: [94°C×1min, 60°C×1min, 72°C×2min] 72°C×10min 4°C forever	Used Triplet Primed PCR for the detection of large expansion on genomic DNA samples.

Kakourou *et al.* (2007)	DMPK: Forward: (CTTCCCAGGCCTGCAGTTTGCCC ATC) Reverse:(GAACGGGGCTCGAAGGGTCCTTGTAGC) APOC2: Forward:(GGCTACATAGCGAGACTCCATCTCC) Reverse:(GGGAGAGGGCAAAGATCGATAAAGC) D19S112: Forward: (GCCAGCCATTCAGTCATTTGAAG) Reverse: (CTGAAAGACACGTCACACTGGT)	DM1-APOC2 duplex PCR: Total=25 µl 0.3µM Primers with DM1 FAM and APOC2 HEX, 0.2 mM dNTP,2.5 µl 10× AmpliTaq buffer (100mM Tris-HCl, pH8.3, 500mM KCl, 15mM $MgCl_2$, 0.01% gelatine),1.5U AmpliTaq Gold Enzyme (PE Applied Biosysytems)	DM1-APOC2 duplex PCR: 95°C×12min, 40 cycles: [94°C×30sec (96°C for 10 cycles), 60°C×45sec, 72°C×45sec] 72°C×10min	The diagnosis in this case was performed using duplex and triplex fluorescent- polymerase chain reaction to amplify DMPK with APOC2 AND D19S112 polymorphic markers thus showing the importance of linked polymorphic markers in PGD-PCR.
		DM1-APOC2-D19S112 triplex PCR: Total=25 µl 0.2 µM DM1F/R,0.3 µM APOC2 and D19S112, 0.2 µM dNTP,2.5 µM High Fidelity Buffer 2 (15mM $MgCl_2$), 1.5U High Fidelity enzyme mix (Roche Diagnostics, UK)	DM1-APOC2-D19S112 triplex PCR: 95°C×2min, 40 cycles: [94°C×15sec (96°C for 10 cycles), 58°C×45sec, 72°C×1min] 72°C×7min	
Kim *et al.* (2008)	FAM102F: (5`-GAACGGGGCTCGAAGGGTCC T TGTAGC-3`) 101R: (5`-CTTCCCAGGCCTGCAGTTTGCCCA TC-3`)	Total-25µl 1xPCR buffer,0.2mM dNTP,2.5U Taq polymerase, 10pM FAM102F and 101R Primers,100ng of DNA	94°C×2min 10 cycles: [94°C×10sec, 65°C×30sec 68°C×3min] 20 cycles: [94°C×10sec, 65°C×30sec 65°C×2min (20sec added/cycle)] 68°C×7min	The PCR products were analyzed by the GenScan analysis program to enable haplotype analysis of DM1 locus in Korean population
Skrzypczak-Zeilinska *et al.* (2009)	Long PCR: Used DMK9003 and DMK11111 (Cheng et al., 1996)	Total-25µl 2.5µl of10× Hot Start Buffer without Mg, 1mM $MgCl_2$,2.0µl of 2.5mM dNTP,0.25U Hot Start polymerase,7% Taq-Stablizer,20% GC - enhancer 0.5pmol PrimerDMK9003 & DMK11111, 20ng DNA	95°C×2mins 30 cycles: [95°C×30sec, 61°C×30sec, 72°C×7min] 72°C×7min	In order to develop a highly sensitive and cost effective molecular approach for DM1 alleles characterization a combination of Long PCR and QF-PCR was used for analysis. Ref1 and Ref2 are used as markers.
	QF PCR: Used Primer 101 and FAM102 (Brook *et al.*, 1992) is used with Ref1For:(AAGCATGCTTCAGCCCAGCCAAGC) Ref 1 Rev:(6-FAM-ATTTCTACAGGCTGGACCCGCTG) (Fitzky *et al.*,1998) Ref 2 For:(CGCCCCGAGTGTCCATGTGTT) Ref 2 Rev:(6-FAM-TACTTCTGCAAGCAGAAAGCTCCCTCC) (Schimmenti *et al.*, 1997)	Total-25µl 2.5µl of Hot Start Buffer without $MgCl_2$,200mM/l Tris HCl, 500mM/l KCl[pH 8.5],1.8mM/ l $MgCl_2$, 2.5µl of 2.5mM dNTP,0.8pM of DM1 Primers 1U Taq polymerase,6% Taq-Stablizer,16% GC – enhancer,80ng DNA	95°C×7mins 14 cycles: [95°C×30sec, 67°C×30sec, (decreasing 1°/cycle) 72°C×45sec] 72°C×5min	
Magnana *et al.* (2010)[42]	Warner *et al.,* 1996	Warner et al., 1996	95°C×7min For P1: 35 cycles: [94°C×1min, 58°C×1min, 72°C×90sec] For P2: 35 cycles: [	Molecular diagnosis for DM1 was performed using a combination of fluorescent PCR and capillary electrophoresis for analysis.

			94°C×1min, 59°C×1min, 72°C×120sec] 5min in ice	
Hamzi *et al.* (2010)[43]	5`- GAAGGGTCCTTCTAGCC GGGAA-3` 5`- CAGAGCAGGGCGTCATG CAGC-3`	Total-25µl 1mM/l $MgCl_2$,50.25mM/l Tris HCl,45mM/l $(NH_4)_2SO_4$,200µM/l dNTP,2.5U Taq pol, 200pM Primer100ng DNA	95°C×10min 30 cycles: [94°C×1min, 60°C×1.5min, 72°C×2min] 72°C×5min	Used rapid PCR method for initial screening of the samples and the positive samples can be confirmed by TP-PCR and Southern Blot.
Radvansky *et al.* (2011)[44]	For forward end: P1F:(5`- GGGGCTCGAAGGGTCCT TGT-3`) P4CAGR: (5`- AGCGGATAACAATTTCA CACA GGACAGCAGCAGCAGCA GCAG-3`) P3:(5`AGCGGATAACAATT TCACACAGGA-3`) P2R:(5`- GGGGCTCGAAGGGTCCT TGT-3`) P4CTGF:(AGCGGATAACA ATTTCACACAGGATGCTG CTGCTGCTGCTGCTG-3`) P3: (5`- AGCGGATAACAATTTCA CACAGGA-3`)	Total-10µl 1×PCR buffer,1.5mM/l $MgCl_2$,1M Betaine,150µM/l dNTP,0.25U Taq polymerase,2pM P1F, 0.5pmolP4CAGF, P4CTGF,1.5pM P3,300ng DNA	95°C×5min 34 cycles: [94°C×1min, 65°C×1min, 72°C×2min]	Used combination of bidirectionally labelled conventional PCR with TP-PCR in both directions for increasing reliability and accuracy of TP-PCR based assay to analyze genomic DNA.
Orpana *et al.* (2012)	FAM-DM3107F:(5`- CTTCCCAGGCCTGCAGT TTGCCCAATCC-3`) DM3108R:(5`- GAACGGGGCTCGAAGGG TCC T TGTAGCC-3`)	Total-50µl 1xPCR buffer,160µM/L dNTP,0.8U Taq polymerase,20pMFAM-DM3107 and DM3108, 20ng of DNA	94°C×10min 20 cycles: [95°C×1min, 68°C×1min (decreasing to 60°C at -0.4°C/cycle)] 72°C×3min 16 cycles: [95°C×1min, 60°C×1min Ramp to 72°C×3mins] 72°C×10min	In case of Heat pulse extension PCR a continuously shifting extension temperature allows extension of large CTG repeats.
	DMKF:(5`- GCCAGTTCACAACCGCTC CGAG CGTGGGTC-3`) DMKR:(5`- ACGCTCCCCAGAGCAGG GCGTCA TGC-3`)	Total-40µl 1xPCR buffer,200µM/L dNTP,1.33U Taq polymerase,100pM DMKf and DMKr,2.25mM/L Betaine and 40ng of DNA	94°C×7min 40 cycles: [95°C×45sec 98°C×10sec 68°C×30sec Ramp to 76°C (30%ramp rate) 21×Ramp to 83°C(30%) 76°C×2 sec]	

[Only the methods giving complete information of the conditions are listed above]